OIPE JC
MAR 11 1999
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group Art Unit 2877 :

Examiner K. P. Hantis :

In re application of :

PATRICK TREADO : CHEMICAL IMAGING SYSTEM

Serial No. 09/064,347 :

Filed April 22, 1998 :

5/24/ Declarat

RECEIVED
MAR 23 PM 1:35

RULE 1.132 DECLARATION OF PATRICK TREADO

I, Patrick Treado, do declare and state as follows:

1. I am the sole inventor of United States Patent Application Serial No. 09/064,347 for a Chemical Imaging System.

2. I have been active in the field of Raman imaging since 1986. Since then, I have made use of a number of approaches for fully utilizing Raman imaging. Since 1991, I have studied the use of tunable filters, including those based on liquid crystals and acousto-optics, used in connection with a Raman imaging system.

3. Persons having ordinary skill in the art of Raman imaging would not have thought to use an Evans Split Element liquid crystal tunable filter (LCTF) in place of traditional liquid crystal filters.

4. The -Evans Split Element filter was invented for color imaging and display which is very different than Raman imaging. Typically, these LCTFs are characterized by high speed, high throughput, broad spectral bandpasses, but reduced out of passband rejection efficiency, and inhomogeneous response across the filter. Requirements of color filters are very

different than the requirements of a Raman imaging spectrometer. To those practiced in the art of color imaging, Raman imaging would be far afield and a non-obvious application. Color imaging relies on high light levels to form images in real-time. Raman imaging is forced to rely on low light levels to form images relatively slowly where most of the light is background fluorescence light and only a small fraction is Raman light. In Raman imaging, high performance instrumentation is employed including high power lasers and ultrasensitive imaging detectors.

5. To those practiced in the art of Raman imaging, color imaging filter characteristics would teach away from their potential use as Raman imaging devices. One of the principal trends in Raman spectroscopy and imaging involved the use of multichannel detectors to capture spectra and images. These multichannel detectors helped to revolutionize traditional Raman spectroscopy which had relied on single element detectors in combination with scanning spectrometers. The operation of color filters teaches away from the art because they rely upon scanning of the spectral dimension in order to build up the color (i.e. spectral) information. To those practiced in the art of Raman spectroscopy, the concept of relying upon a spectral dimension scanning approach goes against recent trends. I recognized that much of the Raman spectrum is redundant and only a minimum number of spectral bands need to be scanned in order to build up sufficient information to analyze a material of interest.

6. Prior to my invention, Evans Split Element LCTFs were never previously envisioned for Raman imaging. Other researchers in the field of Raman imaging were not active in the tunable filter field. The Treado/Morris article appeared in 1989. The Sharp patent was issued in June 1996. The Raman imaging community is small and has several technical forums

for communication where those skilled in the art actively participate. If the invention was obvious it would have been reduced to practice prior to my invention.

7. Prior to my invention, the Evans Split Element filter was thought to not have sufficient spectral resolution for Raman spectroscopy applications. Today, due to my work, it is becoming widely recognized that Evans Split Element LCTFs are a superior technology for Raman imaging when incorporated into a well designed chemical imaging system. However, those practiced in Raman spectroscopy and imaging prior to 1998 would have assessed the performance of Evans Split Element technology based on traditional measures of Raman instrument performance and would likely have rejected the technology. For example, it is widely believed that a Raman spectrometer has to have resolution of at least 4 cm^{-1} in order to be an effective instrument. Applicant has demonstrated superior system performance using an LCTF that has a spectral resolution of 8 cm^{-1}.

8. Based on its wavelength operating range (visible wavelengths) the Evans Split Element filter was thought to not be applicable for Raman imaging of fluorescent samples. To those practiced in the art of Raman spectroscopy, it would be expected that Raman spectrometers could only be effective when background fluorescence (a source of interference when performing a Raman experiment) was not present. Strategies for eliminating background fluorescence have been developed and accepted as essential by the Raman spectroscopy community. These strategies teach away from the use of a Evans Split Element LCTF operating under conditions discussed in the application – with green wavelength laser sources. Namely, that Evans Split Element LCTFs employed in imaging mode can be used to investigate highly fluorescent species using visible laser illumination and detection. I have recognized that the key to imaging fluorescent samples is that the impurities that typically cause fluorescence are often

not homogeneously distributed throughout the sample and are spatially localized within the material. With the imaging capability of the Evans Split Element filter, the spatially resolved fluorescence interference can often be differentiated from the Raman information that is diagnostic of the sample material of interest.

9. To those practiced in the art of solid-state tunable filters, the Evans Split Element filter is relatively slow (msec tuning speed) as a scanning imaging spectrometer when compared to alternative technologies. The Evans Split Element filter is much slower than other tunable filters such as acousto-optic tunable filters (AOTFs) (microsec tuning speed). The use of AOTFs in Raman imaging was pioneered by me and AOTFs represented state of the art technology when I first used them. Because they were state of the art, a perception developed in the Raman imaging community that AOTFs were suitable for Raman imaging despite key limitations, including low spectral resolution and poor imaging performance. To those practiced in the art, AOTFs would have appeared to be superior to Evans Split Element technology on the basis of tuning speed. However, I recognized that tuning speed is a minor consideration in Raman imaging, because overall image acquisition time is dominated by the low light level conditions which require long signal acquisition times (1-30 secs). The subsecond tuning speeds provided by tunable filters, including Evans Split Element filters, are adequate.

10. Narrow bandpass Evans Split Element filter have an inherently low peak transmittance. For example, the filter can transmit 10% of the light presented to it on average. The low peak transmittance teaches away from its use in a low light level imaging technique like Raman imaging. The peak transmittance of the Evans Split Element filter is relatively low compared with competitive, dispersive Raman spectrometer technology such as volume holographic diffraction gratings that produce greater than 50% transmittance. However, what is

not appreciated by those practiced in the art within the Raman community is that the clear aperture of the Evans Split Element LCTF is substantially larger than the clear aperture of even the most efficient dispersive spectrometers. As a result, the total throughput of the Evans Split Element filter is comparable to or better than competitive technology.

11. Liquid crystal optics are susceptible to temperature drift. The Evans Split Element filter spectral performance is susceptible to temperature induced drift. The drift is more significant than dispersive Raman spectroscopy technology. As a result, to those practiced in the art of Raman spectroscopy, the poor temperature drift performance of the technology would teach away from the technology. A drift compensation approach based on capacitance coupled feedback has been effectively employed by the manufacturer of the Evans Split Element filter. The drift compensation approach would increase in complexity with the number of liquid crystal cells employed in the device. As a result, to those practiced in the art of Evans Split Element filter manufacturing, fabricating a multi-element Evans Split Element filter with adequate temperature compensation would have been viewed as impractical. In order to achieve broad tunability (500-750 nm) and narrow spectral bandpass 8 cm^{-1} (0.25 nm @ 500 nm) simultaneously, the Evans Split Element LCTF requires more than 20 liquid crystal elements. Each of the stages that comprises the Split Element liquid crystal filter passes several bandpasses simultaneously. Optimizing the Evans Split Element filter to pass a single passband and reject out of band light is a daunting fabricating challenge, but as it turns out, it can be accomplished on a routine basis.

12. Fabricating an ultra-narrow bandpass Evans Split Element filter would have been viewed as impractical. Evans Split Element liquid crystal filters were invented for color imaging and display applications. In those applications, spectral bandpasses of ten to a

hundred nanometers are typically employed. For Raman imaging, filters with bandpasses of less than 10 cm^{-1} are required. To those practiced in the art of Evans Split Element filter technology, it would appear impractical to fabricate such a device. Given that the technology had been envisioned for color imaging applications, to those practiced in the art of Raman spectroscopy, it would have appeared impractical that such a device could be fabricated to produce such a narrow spectral bandpass. In essence, the manufactured technology taught away from the art.

13. The inhomogeneous response of the Evans Split Element filter due to off-axis effects teaches away from the use of the device for Raman imaging in two ways. First, inhomogeneous distribution of transmittance in the Evans Split Element filter teaches away from the art. The inhomogeneous transmittance is attributed to an off-axis effect that has been well characterized and previously described (Title and Rosenberg, 1979; Deng, Ai and Wang, 1997). To those practiced in the art of Raman imaging, Raman imaging contrast is based on local intensity variations across the image field of view. Inhomogeneous transmittance would present a real challenge because the Raman intensity images would have superimposed an inhomogeneous transmittance pattern due to the Evans Split Element filter that cannot be compensated using traditional Raman image data processing schemes. I recognized that the inhomogeneous transmittance does not affect the spectral performance of the Evans Split Element filter and Raman imaging data processing of the spectral patterns is an effective approach.

14. The software used in my invention employs a data processing means that corrects for brightness differences - based on multivariate image analysis. Specifically, Raman chemical images that are displayed by my invention are based on spectral shapes and not based

on spectral intensity. As a result, local intensity variations that arise from the inhomogeneous transmittance are compensated.

15. Second, I recognized that the inhomogeneous response of the Evans Split Element filter also manifests itself as inhomogeneous out-of-band rejection efficiency across the Evans Split Element filter. The inhomogeneous out-of-band rejection efficiency teaches away from the art. It is widely recognized that typical Raman applications are background limited. This means that the interference species present in the sample limit the ability to probe the sample. A typical interference is fluorescence background due to impurities in the sample. Another interference is the laser illumination stray light that appears in the microscope due to inefficient mirrors and optical filters. My invention employs carefully placed intermediate apertures that reduce the laser illumination stray light component.

16. However, when a fluorescence background is present, the inhomogeneous out-of-band rejection efficiency cannot be compensated with intermediate apertures. I recognized that the use of spectral patterns recognition approach that compensates for inhomogeneous transmittance also compensates for inhomogeneous out-of-band rejection efficiency. The software used in my invention employs a data processing means that corrects for out-of-band rejection differences - based on multivariate image analysis. As a result, Raman chemical images displayed by my invention that are based on spectral shapes and local intensity variations that arise from the inhomogeneous out-of-band rejection efficiency are compensated.

I declare that the foregoing is true and correct, that all statements made on information and belief are believed to true, and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine,

imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any false statements may jeopardize the validity of this Declaration and the above-identified patent.

Pat J. Treado

Patrick Treado